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Effects of spontaneous heating on forage protein fractions and in situ disappearance kinetics of crude protein for alfalfa-orchardgrass hays packaged in large round bales[1]

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ABSTRACT

During 2006 and 2007, forages from 3 individual hay harvests were used to assess the effects of spontaneous heating on concentrations of crude protein (CP), neutral detergent insoluble CP (NDICP), acid detergent insoluble CP (ADICP), and in situ disappearance kinetics of CP and NDICP for large round bales of mixed alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.). Over the 3 harvests, 96 large round bales were made at preset bale diameters of 0.9, 1.2, or 1.5 m and at moisture concentrations ranging from 9.3 to 46.6%. Internal bale temperatures were monitored daily during an outdoor storage period. The change in concentrations of NDICP (poststorage − prestorage) increased with heating degree days (HDD) >30°C in a relationship best explained with a nonlinear model $\{Y = 24.9 - [22.7 \times (e^{-0.000010 \times x \times x})]; R^2 = 0.892\}$ that became asymptotic at +24.9 percentage units of CP, thereby indicating that NDICP increases rapidly within bales that heat spontaneously. When maximum internal bale temperature (MAX) was used as the independent variable, the best regression model was quadratic and the coefficient of determination was still relatively high ($R^2 = 0.716$). The change in concentrations of ADICP (poststorage − prestorage; ΔADICP) also increased with HDD and was best fitted to a nonlinear model $\{Y = 14.9 - [15.7 \times (e^{-0.0000019 \times x \times x})]\}$ with a very high coefficient of determination ($R^2 = 0.934$). A similar quartic response was observed for the regression of ΔADICP on MAX ($R^2 = 0.975$). Increases in ΔADICP as a result of heating (HDD or MAX) were paralleled by concurrent increases in hemicellulose at relatively low increments of heating, but the inverse relationship was observed as hemicelluloses likely became reactive and concentrations decreased in more severely heated hays. Changes in ruminal disappearance rate of CP were best fitted to cubic models for regressions on both HDD ($R^2 = 0.939$) and MAX ($R^2 = 0.876$); these changes represented an approximate 50% rate reduction in severely heated hays relative to prestorage controls. Within ranges of heating most commonly encountered under field conditions, changes in rumen-degradable protein decreased in a primarily linear relationship with HDD or MAX. However, the mean change in rumen-degradable protein for the 4 most severely heated hays was only −2.6 percentage units of CP, which represents a minimal reduction from prestorage controls and is far less than the maximum of −7.9 percentage units of CP observed with less-severe heating. Interpretation of these results was complicated by poor recovery of NDICP from our most severely heated hays following machine rinsing of 0-h Dacron bags; theoretically, and by definition, this unrecovered pool of NDICP is assumed to be entirely degradable in the rumen. It remains unclear whether these responses could be corroborated in vivo or by other analytical techniques, or whether the magnitude of HDD or MAX for our most severely heated hays exceeds the reliable limits for estimating RDP via in situ methodology.
Key words: acid detergent insoluble crude protein, crude protein disappearance kinetics, neutral detergent insoluble crude protein, spontaneous heating

INTRODUCTION

Harvests of alfalfa and other dairy-quality hays are frequently complicated by poor drying conditions or the threat of unexpected rainfall events, each of which potentially places these valuable crops at risk. As a result, producers often are forced to choose between baling their hay crop before adequate desiccation has occurred or subjecting their wilting hay to rain damage. Spontaneous heating is a common phenomenon observed within hays baled without proper desiccation; this process

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occurs as plant sugars are respired into CO_2, water, and heat by microorganisms associated with the hay (Rotz and Muck, 1994). Traditionally, hay research has used small (approximately 45-kg) rectangular bales as the experimental model, in part because they are easy to replicate. As a result, a representative set of treatments can be evaluated using only a limited land area. The threshold moisture concentration for acceptable storage for this type of hay package is approximately 20% (Collins et al., 1987). However, the cost and limited availability of labor necessary to handle conventional rectangular hay bales has forced many hay producers to consider larger bale types. Generally, these larger hay packages are more prone to heat spontaneously and require a reduced threshold moisture concentration for acceptable storage. In addition, large round bales can exhibit far greater quantitative measures of spontaneous heating, such as heating degree days (**HDD**) >30°C or maximum internal bale temperature (**MAX**), than are observed typically for small rectangular hay bales (Coblentz and Hoffman, 2009a). As a result of these factors, in-depth evaluations of the effects of spontaneous heating on CP composition and associated kinetic estimates of ruminal disappearance of CP for heated forages packaged within large round or square bales have been limited.

Historically, the CP recovered within insoluble acid-detergent fiber (**ADICP**) residues has been suggested to be a relatively sensitive indicator of heating in forages, and concentrations of ADICP frequently increase linearly with measures of spontaneous heating in hay (Coblentz et al., 1996, 2000). Concentrations of ADICP are believed to increase via formation of Maillard or nonenzymatic browning products that are produced via condensation of sugars with AA, subsequently yielding polymers possessing many of the physical properties of lignin (Van Soest, 1982). A generation ago, Goering et al. (1973) determined that this reaction in forages is exacerbated by effective heating period, temperature, and the moisture concentration within the hay. This reaction is important because ADICP is presumed to exhibit very low bioavailability in ruminants (Van Soest and Mason, 1991; Licitra et al., 1996). In practice, 2 in vivo studies (Broderick et al., 1993; McBeth et al., 2001) suggest this premise is true for ADICP in its native form, but bioavailability may improve somewhat as concentrations of ADICP increase as a consequence of spontaneous heating. McBeth et al. (2001) reported that apparent digestibilities of ADICP for bermudagrass [*Cynodon dactylon* (L.) Pers.] hays incurring 5 to 401 HDD during storage increased linearly from −1.7 to 42.3% when offered to wether lambs. Similarly, Broderick et al. (1993) found a negative apparent digestibility of ADICP (−12.2%) for dairy cow diets containing unheated alfalfa hay, but a positive digestibility (35.8%) for diets containing steam-heated hays. Although ADICP is commonly used as an indicator of heat damage, neutral detergent insoluble CP (**NDICP**) also is known to increase linearly in response to spontaneous heating within bermudagrass hays packaged in small rectangular bales (Coblentz et al., 2000; Turner et al., 2002). Across both of these studies, the relationships between NDICP and HDD were characterized by relatively high coefficients of determination ($r^2 \geq 0.612$). Quantification of NDICP is important within the Cornell Net Carbohydrate and Protein System because CP that is insoluble in neutral detergent but soluble in acid detergent comprises the B_3 protein fraction that is assumed to retain bioavailability but also is relatively resistant to ruminal degradation (Sniffen et al., 1992). In part, the B_3 fraction may increase with spontaneous heating because B_2 proteins that are soluble in neutral detergent often denature with heating, thereby rendering them increasingly insoluble (Licitra et al., 1996). Quantification of NDICP also is required to estimate energy density by the summative approach (Weiss et al., 1992; NRC, 2001).

Many current nutritional models (Sniffen et al., 1992; NRC, 1996, 2001) for ruminants require estimation of the percentage of RDP. For alfalfa, rates of ruminal CP disappearance ($\mathbf{K_d}$) are known to be extremely rapid (Hoffman et al., 1993; Vanzant et al., 1996), which contributes to extensive estimates of RDP (NRC, 2001) and inefficient use of this forage protein. As a result, considerable research effort has been directed at assessing techniques that potentially reduce K_d and RDP for alfalfa hays and silages. The baling and storage process alone offers some benefit; mean estimates of K_d and RDP have been reduced from 0.171 to 0.075/h and 76 to 60% of CP, respectively, in comparisons of standing alfalfa forage with baled hays (Broderick et al., 1992). External heat treatments, applied as forced air or via steam, reduced both K_d and RDP in shredded and unshredded alfalfa hays (Yang et al., 1993), but these practices also reduce forage energy density, thereby requiring additional compensation with concentrate feeds to maintain milk production (Broderick et al., 1993). Heat generated spontaneously during bale storage also has reduced estimates of K_d and RDP within both alfalfa (Coblentz et al., 1997) and bermudagrass hays (McBeth et al., 2003). For alfalfa, the negative linear relationship between K_d and HDD was especially close ($r^2 = 0.856$) and resulted in a 50% reduction in K_d. Similarly, RDP within these hays decreased linearly by 0.018 percentage units of CP per HDD, also exhibiting a relatively high coefficient of determination ($r^2 = 0.615$). However, these studies used only small rectangular or model baling systems in which heating characteristics were limited to a much narrower range than those attainable in larger bale packages. Therefore, our objectives were to relate changes in concentrations of CP components and characteristics of in situ CP or NDICP disappearance to measures of spontaneous heating for large round bales of alfalfa-orchardgrass hay described previously (Coblentz and Hoffman, 2009a) using linear and nonlinear regression techniques.

MATERIALS AND METHODS

Field Procedures

Considered collectively, this project comprised 3 independent hay harvests conducted on the same 8.2-ha field site during 2006 and 2007. Establishment of forages, soil fertility, harvest management, storage procedures, temperature measurements, and pre- and poststorage sampling procedures have been described in detail previously (Coblentz and Hoffman, 2009a) and have been summarized thoroughly in a related communication (Coblentz and Hoffman, 2009b). Therefore, only a brief, general overview of these procedures is provided.

An 8.2-ha field site comprising a mixture of Phabulous II alfalfa and Extend orchardgrass was established on April 14, 2004 near Stratford, Wisconsin. The 3 independent hay harvests included harvest and baling of a second (low moisture; **LM**) and third (high moisture; **HM**) cutting during 2006, as well as the harvest and baling of a second cutting during 2007 (intermediate moisture; **IM**). Within each hay harvest, the treatment structure was similar; generally, bales were packaged in factorial arrangements of bale diameter (1.5, 1.2, or 0.9 m) and various concentrations of moisture. Each combination of bale moisture and bale diameter was replicated in 3 experimental field blocks that were based on field topography (slope). For the LM, IM, and HM harvests, prestorage bale moistures for interactive treatment combinations ranged from 9.3 to 17.3%, 16.8 to 24.2%, and 26.7 to 46.6%, respectively. Respective dry weight percentages of alfalfa in LM, IM, and HM harvests were 91, 76, and 68%; similarly, orchardgrass composed 9, 22, and 31% of each sward. During each harvest, forage was mowed and conditioned (model 8830; J. I. Case Co., Racine, WI), adjacent rows were gathered with a bifold rake, and hay was packaged with a Ford-New Holland round baler (model BR 740A; CNH America LLC, Racine, WI). All bales were tied with 2 revolutions of net wrap, positioned on wooden pallets located outdoors, and then monitored daily for 1) HDD, computed as the summation of the daily increments by which the internal bale temperature was greater than 30°C, and 2) MAX. All bales were sampled (Uni-Forage Sampler; Star Quality Samplers, Edmonton, Alberta, Canada) twice. One sampling occurred immediately after baling, and another after bales exhibited no further evidence of spontaneous heating. For the poststorage sampling, hay samples were obtained from the surface layer (0.15 m deep) and then the bale core. All hay samples were dried to constant weight under forced air (50°C), ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) equipped with either a 1- or 2-mm screen, and then retained for laboratory evaluation of CP, NDICP, ADICP, and ruminal in situ disappearance kinetics of CP and NDICP.

Laboratory Analyses

Portions of each hay ground through a 1-mm screen were analyzed for total N by a macro-Kjeldahl procedure (AOAC, 1998; method 988.05). Concentrations of NDICP and ADICP were determined similarly following nonsequential extraction in neutral and acid detergent, respectively. The NDF solution contained no sodium sulfite or heat-stable α-amylase, and residual CP following extractions in either neutral or acid detergent was determined by the identical macro-Kjeldahl procedure described previously. Concentrations of NDICP and ADICP then were reported as a percentage of total CP.

In Situ Incubation Procedures

Selection of Hays. Eighteen interactive (bale moisture × bale diameter) treatments were selected from the HM (10 treatments) and IM (8 treatments) harvests for in situ analysis. All poststorage hays evaluated in situ consisted of forage from the bale core only, and each hay treatment was composited over the 3 field replications (bales) before conducting in situ evaluations. The 18 treatments were selected to provide the best possible distribution across the entire heating continuum represented by IM and HM harvests. Composites of prestorage samples generated from the 10 hays selected from the HM harvest and from the 8 hays selected from the IM harvest also were evaluated as controls (no heating). Although hays from the LM harvest were evaluated for concentrations of CP, NDICP, and ADICP, they were not considered for in situ analysis. This was a procedural compromise necessitated by the upper limit of about 20 hays that could be evaluated simultaneously using in situ techniques. We chose to eliminate hays from the LM harvest because 1) they did not broaden the range of accumulated HDD (25–343 HDD) relative to the IM harvest (19–506 HDD; Coblentz and Hoffman, 2009a) and 2) they did not improve the distribution of HDD within this range.

In Situ Incubation Procedures. The general methodology for in situ evaluation of heated forages has been detailed previously (Coblentz and Hoffman, 2009a,b); therefore, in situ procedures will be outlined only in brief. Two nonlactating, ruminally cannulated Holstein cows (937 ± 35.4 kg) were housed in individual 4.3 × 8.5-m pens and offered a basal diet consisting of shredded alfalfa–quackgrass hay (14.0% CP, 50.8% NDF, and 36.0% ADF), ground corn, and trace mineralized salt. The basal diet was offered at 0900 and 1500 h daily in equal portions at a cumulative rate of 1.35% of BW. On an as-fed basis, the diet consisted of 90.3% alfalfa–grass hay, 8.9% ground corn, and 0.8% trace mineralized salt. Individual pens had concrete floors that were bedded with wood shavings, and fresh water was available in each pen for ad libitum intake. Cannulations (protocol #A-1307) and care of the cows (protocol #A-1339) were approved by the Research Animal Resources Center of the University of Wisconsin. Cows were adapted to the basal diet for 10 d, and then hays were evaluated during two 4-d experimental periods. A 3-d recovery period was allowed between periods 1 and 2.

Five-gram samples of each dried, ground (2-mm screen) hay were sealed in 10 × 20-cm Dacron bags (50 ± 10-μm pore size; Ankom Technology Corp., Fairport, NY) and then suspended in the ventral rumen for 3, 6, 9, 12, 24, 36, 48, 72, or 96 h. Prior to ruminal incubation, all Dacron bags were placed in 35 × 50-cm mesh bags and soaked in tepid water (39°C) for 20 min. After removal from the rumen, Dacron bags were washed immediately in cold water in a top-loading washing machine (10 total rinse cycles, 1 min of agitation and 2 min of spin per rinse; Coblentz et al., 1997; Vanzant et al., 1998). Four additional Dacron bags per experimental forage were preincubated and rinsed without ruminal incubation, thereby creating a 0-h incubation time. After machine rinsing, residues were dried at 50°C and then equilibrated with the atmosphere in the laboratory before quantifying residual DM (Vanzant et al., 1996). To determine disappearance kinetics of CP, the concentration of CP within each air-equilibrated residue was determined by a rapid combustion procedure (AOAC, 1998; method 990.03; Elementar Americas Inc., Mt. Laurel, NJ). Concentrations of NDICP for each residue were quantified similarly following nonsequential extraction of a 0.5-g subsample in neutral detergent containing no sodium sulfite or heat-stable α-amylase.

Percentages of CP or NDICP remaining at each incubation time were fitted to the nonlinear regression model of Mertens and Loften (1980) using PROC NLIN of SAS (SAS Institute, 1990), which partitioned CP or NDICP into 3 fractions based on relative susceptibility to ruminal disappearance. Fractions A, B, and C were defined as the portions of CP or NDICP disappearing at a rate too fast to measure, disappearing at measurable rate, or unavailable in the rumen, respectively. Fractions B and C, lag time, and K_d were estimated directly from the nonlinear regression model. Fraction A was then calculated as 100% − (B + C), and effective ruminal disappearance of CP or NDICP was calculated as A + B × $[K_d/(K_d + Kp)]$ (Ørskov and McDonald, 1979), where Kp is passage rate (0.06/h; Hoffman et al., 1993). Before calculating disappearance kinetics, a subset (n = 73) of in situ residues selected with representation from all cows, forages, and incubation periods was analyzed for concentrations of purines by the method of Zinn and Owens (1986) to assess levels of microbial contamination. Purine concentrations within in situ residues were found to be negligible (overall mean = 0.19 ± 0.093% of DM), and no corrections for microbial contaminant N were made before calculating kinetics of ruminal CP disappearance.

Regressions of CP, NDICP, and ADICP on HDD and MAX

Initially, data for each individual harvest (LM, IM, and HM) were analyzed by independent ANOVA (data not shown). However, the randomized fixed effects (bale moisture and diameter) evaluated throughout these hay harvests affected CP composition and ruminal CP or NDICP disappearance primarily through their close relationship with heating characteristics that were summarized thoroughly in a previous report (Coblentz and Hoffman, 2009a). Therefore, to simplify the presentation of results, the ANOVA for each individual harvest has been omitted and all data are pooled and analyzed collectively by direct regression on HDD or MAX.

Prior to conducting regression analyses, poststorage concentrations of CP, NDICP, and ADICP from the bale core were normalized as a simple mathematical difference resulting from storage (poststorage − prestorage; **ΔCP**, **ΔNDICP**, and **ΔADICP**, respectively), where positive and negative values indicate increased or decreased concentrations, respectively. Normalization of data was required to account for minor differences in prestorage concentrations of CP, NDICP, and ADICP (Table 1) across the 3 harvests. These baseline-adjusted response variables were then regressed against HDD and MAX using nonlinear regression models (PROC NLIN; SAS Institute, 1990) of the general form $Y = b \times (e^{-kx}) - a$ if ΔCP, ΔNDICP, or ΔADICP became negative with spontaneous heating, or $Y = a - (b \times e^{-kx})$ if they became positive. For these model assessments, k was the rate constant, x was the independent variable (HDD or MAX), and a and b were param-

Table 1. Concentrations of CP, neutral detergent insoluble crude protein (NDICP), and acid detergent insoluble crude protein (ADICP) summarized from 96 round bales of alfalfa-orchardgrass hay from 3 harvests[1] made during 2006 and 2007 at Marshfield, Wisconsin

	Prestorage		Poststorage surface		Poststorage core			
Item	Mean[2]	SE[3]	Mean[2]	SE[3]	Mean[2]	Maximum[4]	Minimum[5]	SE[6]
CP, %								
HM	19.3	0.11	18.5	0.16	19.7	21.7	19.2	0.69
IM	16.6	0.12	17.7	0.13	18.1	18.9	17.3	0.43
LM	18.4	0.13	18.6	0.23	19.2	19.9	18.5	0.50
NDICP, % of CP								
HM	18.4	0.38	38.9	0.43	42.7	47.6	36.3	2.26
IM	21.9	0.34	32.3	0.86	32.9	41.9	22.9	2.78
LM	21.1	0.60	31.0	0.80	30.4	39.2	22.5	3.08
NDICP, % of DM								
HM	3.5	0.08	7.5	0.09	8.5	9.3	7.4	0.37
IM	3.6	0.05	5.7	0.15	5.9	7.3	4.2	0.50
LM	3.9	0.10	5.8	0.16	5.9	7.8	4.3	0.73
ADICP, % of CP								
HM	5.6	0.18	12.4	0.29	15.3	21.4	5.9	1.47
IM	7.3	0.30	8.7	0.19	7.9	9.2	6.2	0.71
LM	5.9	0.32	7.7	0.45	6.4	7.7	5.3	0.46
ADICP, % of DM								
HM	1.1	0.05	2.4	0.05	3.1	4.2	1.2	0.32
IM	1.2	0.05	1.5	0.03	1.4	1.6	1.1	0.12
LM	1.1	0.06	1.4	0.08	1.2	1.5	1.0	0.10

[1]Harvest: HM = high moisture (26.7–46.6%), IM = intermediate moisture (16.8–24.2%), and LM = low moisture (9.3–17.3%). Number of interactive treatments during each harvest: HM = 13, IM = 12, and LM = 7. [Harvest HM contained 1 baling treatment made at 26.7% moisture at the 0.9-m bale diameter only, whereas harvest LM contained a dry control made at 9.3% moisture and at the 1.2-m bale diameter only. These additional treatments were made at only 1 diameter because insufficient forage was available to complete the entire factorial arrangement of treatments (bale diameters) at these moisture concentrations. Each interactive treatment represents the mean of 3 bales.] Total number of bales made per harvest: HM = 39, IM = 36, LM = 21.

[2]Overall mean of all interactive bale moisture × bale diameter treatments.

[3]Standard error of the overall mean of all interactive bale moisture × bale diameter treatments.

[4]Maximum value across all interactive treatments.

[5]Minimum value across all interactive treatments.

[6]Standard error of the interactive mean.

eters determined directly by the regression model. For nonlinear regression models, the independent variables (HDD or MAX) also were squared in an attempt to improve fit. Four other polynomial regression models assessing linear, quadratic, cubic, and quartic responses to HDD or MAX were evaluated by PROC REG of SAS (SAS Institute, 1990).

The selection of the most appropriate model for kinetic characteristics of ruminal CP or NDICP disappearance was assessed similarly. Fractions A, B, and C, as well as lag time, K_d, and effective degradability of CP, were normalized across hay harvests by expressing each CP kinetic parameter as a mathematical (baseline-adjusted) difference resulting from storage (poststorage − prestorage; **ΔA**, **ΔB**, **ΔC**, **ΔLAG**, $\mathbf{\Delta K_d}$, and **ΔRDP**, respectively). Respective kinetic parameters for ruminal NDICP disappearance were normalized in an identical manner ($\mathbf{\Delta A_{ND}}$, $\mathbf{\Delta B_{ND}}$, $\mathbf{\Delta C_{ND}}$, $\mathbf{\Delta LAG_{ND}}$, $\mathbf{\Delta K_{dND}}$, and $\mathbf{\Delta RDP_{ND}}$, respectively). Generally, selection of the most appropriate model was based on the greatest coefficient of determination (r^2 or R^2); however, polynomial regression models were not selected if the coefficient or slope for the highest-ordered term did not differ from 0 or if the model included an illogical inflection in the regression curve that could not be explained biologically. Regression techniques used in this project are meant only to describe responses as a function of HDD and MAX; they are not necessarily rigorous enough to extrapolate beyond the context of this data set or to serve as a direct basis for estimating values for unknown forages.

RESULTS AND DISCUSSION

Protein Components

Concentrations of CP, NDICP, and ADICP for the HM, IM, and LM harvests are summarized in Table 1. Data include concentrations of CP, NDICP, and ADICP from samples obtained before storage, as well as those from the 0.15-m surface layer and the bale core after the cessation of spontaneous heating (poststorage).

CP. Changes in ΔCP that occurred during storage were related to both measures of spontaneous heating, HDD and MAX, in simple linear relationships characterized by negative slopes but poor coefficients of determination ($r^2 \geq 0.122$; Figures 1A and 1B for HDD and MAX, respectively). Overall, the mean ΔCP for all 32 baling treatments was 1.0 ± 0.67 percentage units, thereby indicating that bale storage, or spontaneous heating, or both exhibited a concentrating effect on CP. This response has been attributed to a short-term preferential oxidation of carbohydrates from the hay that increases concentrations of CP indirectly (Rotz and Muck, 1994). Previously, Broderick et al. (1992) reported a mean increase of 2.6 percentage units of CP throughout 15 trials comparing baled hay and standing alfalfa forage. Similar responses have been reported for bermudagrass hays packaged in small rectangular bales (Coblentz et al., 2000; Turner et al., 2002); these responses included a positive linear association between concentrations of CP and MAX (range = 40.2–61.8°C), but not with HDD (Coblentz et al., 2000). Other studies (Montgomery et al., 1986) have reported relatively stable concentrations of CP within large round bales of alfalfa-orchardgrass hay during storage; however, some of these inconsistencies may be related to storage time. For long-term storage (6–9 mo), losses of carbohydrates slow over time, but CP may be lost at a continuing rate of approximately 0.25 percentage units of CP per month through volatilization of ammonia or other N compounds (Rotz and Muck, 1994).

NDICP. Traditionally, NDICP has not been used as an indicator of heat damage to forage proteins, although it is made up (in part) by Maillard products formed as a consequence of spontaneous heating and has been known since early work (Goering et al., 1973) to increase with heating. Despite limited attention, NDICP may have considerable relevance because CP that is insoluble in neutral detergent but soluble in acid detergent is assumed to be resistant to ruminal degradation but may retain bioavailability within the ruminant (Sniffen et al., 1992). For the 32 heated hays in the present study, concentrations of NDICP ranged from 18.4 to 21.9% of CP immediately after baling and from 22.5 to 47.6% of CP in samples obtained from the bale core immediately after storage (Table 1). The relationship between ΔNDICP and HDD (Figure 2A) was best fitted to a nonlinear model that became asymptotic at +24.9 percentage units of CP, when approximately 775 HDD were accumulated. The associated coefficient of determination was high ($R^2 = 0.892$) and was maximized by squaring the independent variable (HDD). The regression of ΔNDICP on MAX (Figure 2B) also increased in a nonlinear pattern but was best fitted to a quadratic model with a somewhat poorer coefficient of determination ($R^2 = 0.716$). Previously, concentrations of NDICP were observed to be related linearly to measures of spontaneous heating within bermudagrass hays (Coblentz et al., 2000; Turner et al., 2002). The linear nature of those responses, which were conducted with small rectangular bales that did not accumulate more than 327 HDD based on a 35°C threshold, is not necessarily in contrast with the curvilinear responses noted in the present study. For instance, arbitrarily limiting our regression of ΔNDICP on HDD to baling treatments accumulating a comparable number of HDD (<400) yielded a significant ($P \leq 0.001$) linear relationship (Y = 0.049 x + 0.5; n = 19; $r^2 = 0.781$; data not shown).

ADICP. Mean concentrations of ADICP across the LM, IM, and HM hay harvests ranged from 5.6 to 7.3% of CP before storage, which is consistent with other reports for ADICP in its native form within alfalfa forages (Hoffman et al., 1993; Coblentz et al., 1998). After storage, concentrations of ADICP ranged from 5.3 to 21.4% of CP (Table 1); the greatest concentrations of ADICP were approximately 3 times those reported in other studies with heated alfalfa hays packaged in small rectangular bales (Collins et al., 1987; Coblentz et al., 1996), and doubled the traditional threshold of 10% of CP used frequently to define severely heat-damaged hays.

Regressions of ΔADICP on HDD (Figure 3A) and MAX (Figure 3B) both exhibited curvilinear responses that included relatively slow accumulations of ADICP over modest heating increments (<400 HDD or 55°C MAX) followed by rapid increases thereafter. For the regression on HDD, data were best fitted to a nonlinear model in which the independent variable was squared to improve fit. Unlike the regression of ΔNDICP on HDD, ΔADICP continued to increase over almost the entire range of HDD, and the response curve did not became asymptotic until approximately 1,675 HDD were accumulated, where ΔADICP = +14.9 percentage units of CP. In contrast, a quartic response was observed with MAX as the independent variable. However, the relationship was similar to that described for HDD; ΔADICP increased slowly when MAX was <55°C but then accumulated at a rapid rate thereafter. Coefficients of determination were extremely high ($R^2 \geq 0.934$) for both regressions, thereby corroborating a close, largely cause-and-effect relationship between ADICP and heating that has been established previously within model systems (Goering et al., 1973; Middleton and Thomas, 1983).

The high R^2 statistics observed in this study are consistent with other studies relating ADICP and measures of heating for small rectangular bales of alfalfa (Coblentz et al., 1996) and bermudagrass (Coblentz et al., 2000). When small rectangular bales have been used as the experimental model, the relationship between ADICP and measures of heating has been both positive and linear (Coblentz et al., 1996; Coblentz et al., 2000; Turner et al., 2002). Similarly, Collins et al. (1987) reported positive linear relationships between ADICP and initial moisture concentration (mean = 22.5%) for both small rectangular and large round bales of alfalfa. Moisture content at baling is the major factor influencing temperature development, DM loss, and associated deleterious changes in stored hays (Rotz and Muck, 1994) and can be related directly to the heating characteristics observed within hay bales (Coblentz and Hoffman, 2009a). Our curvilinear responses illustrated in Figures 3A and 3B differ from the linear responses observed in previous studies, but they are ultimately similar to

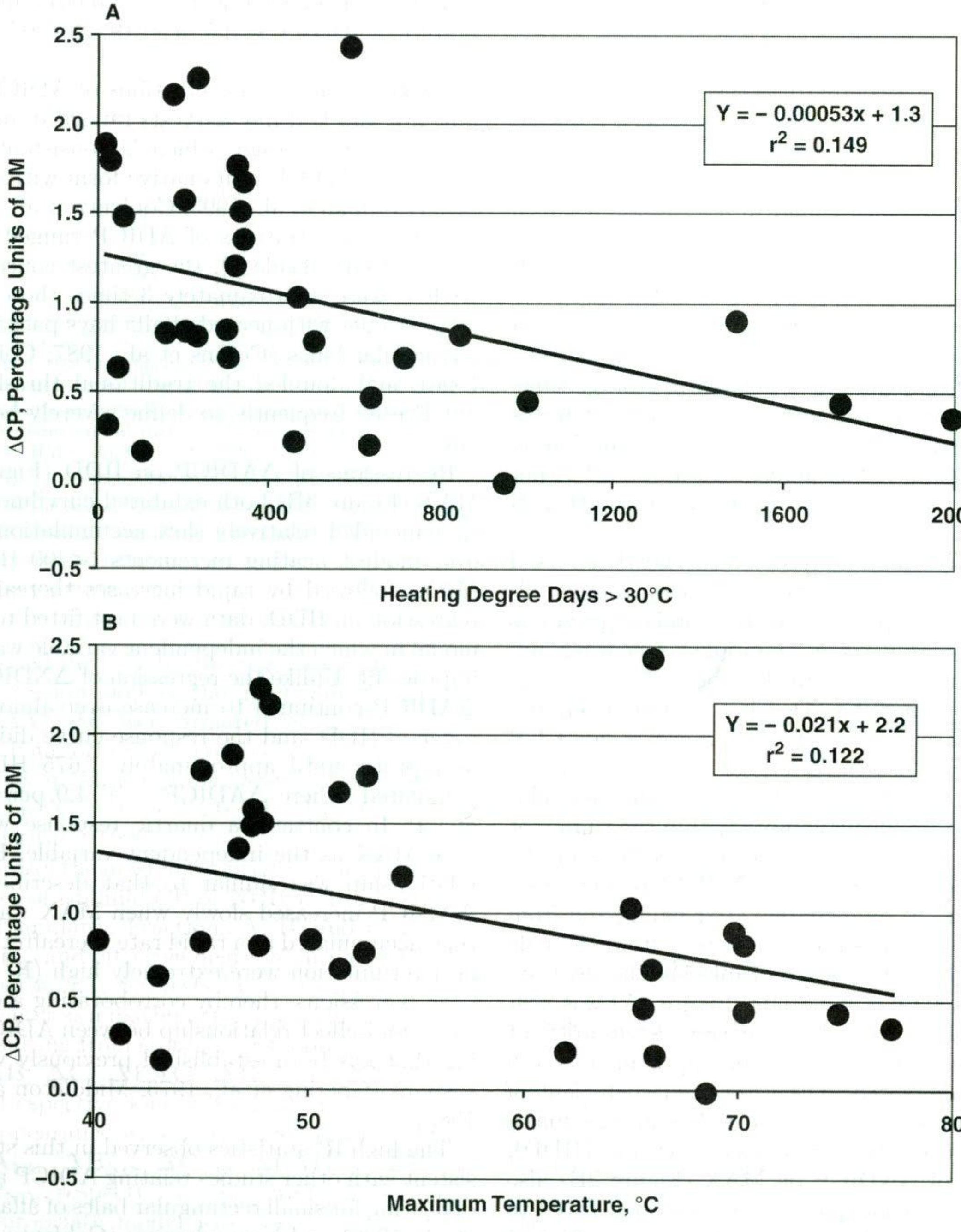

Figure 1. Nonlinear regressions of the changes in concentrations of CP (poststorage − prestorage; ΔCP) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage concentration of CP (weighted on the basis of the number of treatments from the low-moisture, intermediate-moisture, and high-moisture harvests) was 18.1%, which corresponds generally to ΔCP = 0 on the y-axis.

response curves relating ADICP and externally applied heat treatment (40–100°C for 24 h) for orchardgrass hays hydrated artificially to 53% moisture in glass jars (Goering et al., 1973). Furthermore, those authors also reported sharply decreasing concentrations of hemicellulose concomitant with increases in ADICP, and an associated negative correlation coefficient (−0.47; $P < 0.01$) summarized over several laboratory-scale studies. Hemicellulose and soluble carbohydrates, particularly sucrose, are thought to be among the most reactive carbohydrates involved in the Maillard reaction (Van Soest, 1982; Van Soest and Mason, 1991). Under field

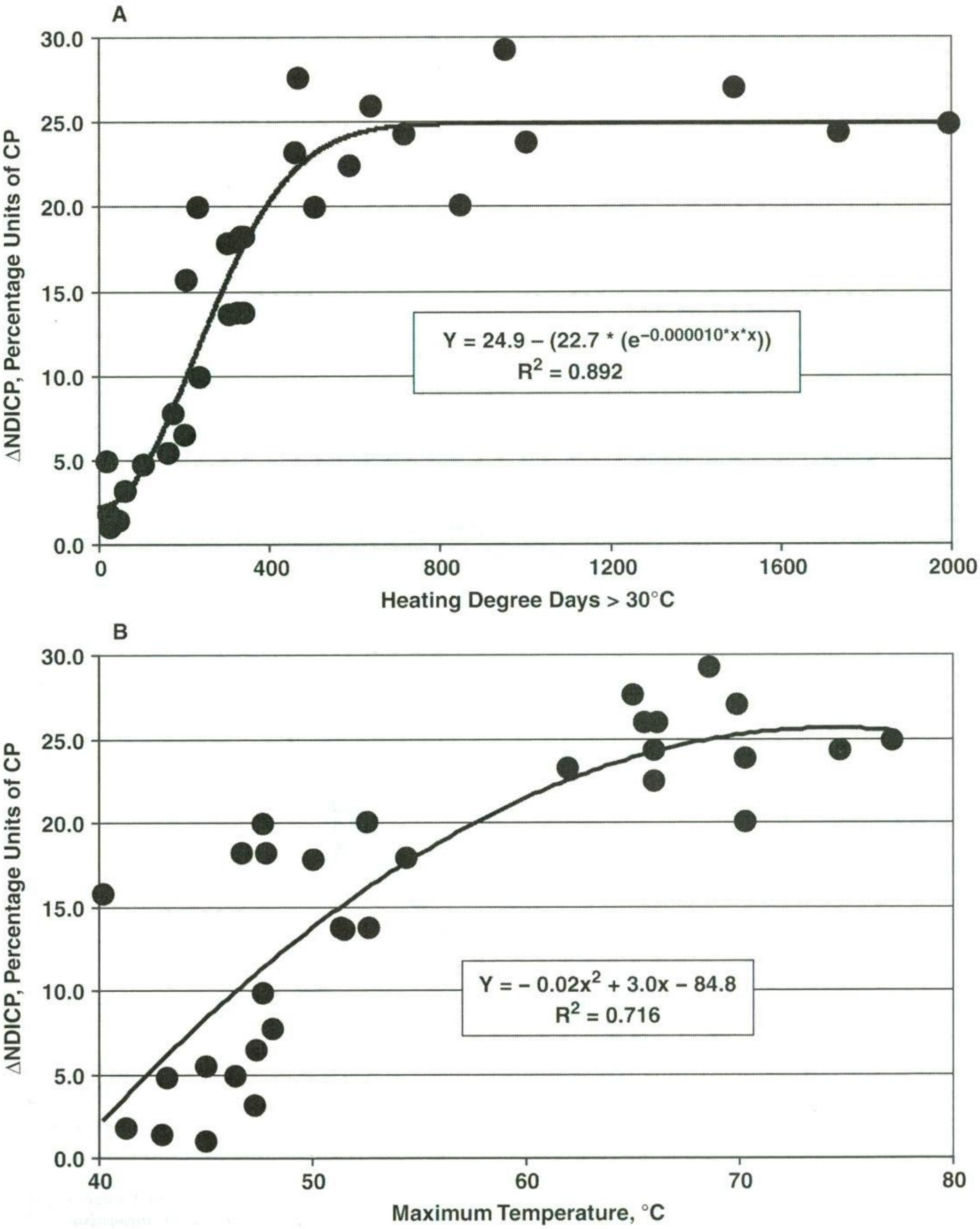

Figure 2. Regressions of the changes in concentrations of neutral detergent insoluble CP (poststorage − prestorage; ΔNDICP) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage concentration of NDICP (weighted on the basis of the number of treatments from the low-moisture, intermediate-moisture, and high-moisture harvests) was 20.3% of CP, which corresponds generally to ΔNDICP = 0 on the y-axis.

conditions, this inverse relationship between hemicellulose and ADICP has not always been observed in hays; Coblentz et al. (2000) reported linear increases in concentrations of both hemicellulose and ADICP within heated bermudagrass hays packaged in small rectangular bales incurring between 25 and 327 HDD during storage. Although not analyzed statistically, increased ADICP was not accompanied by sharp reductions in

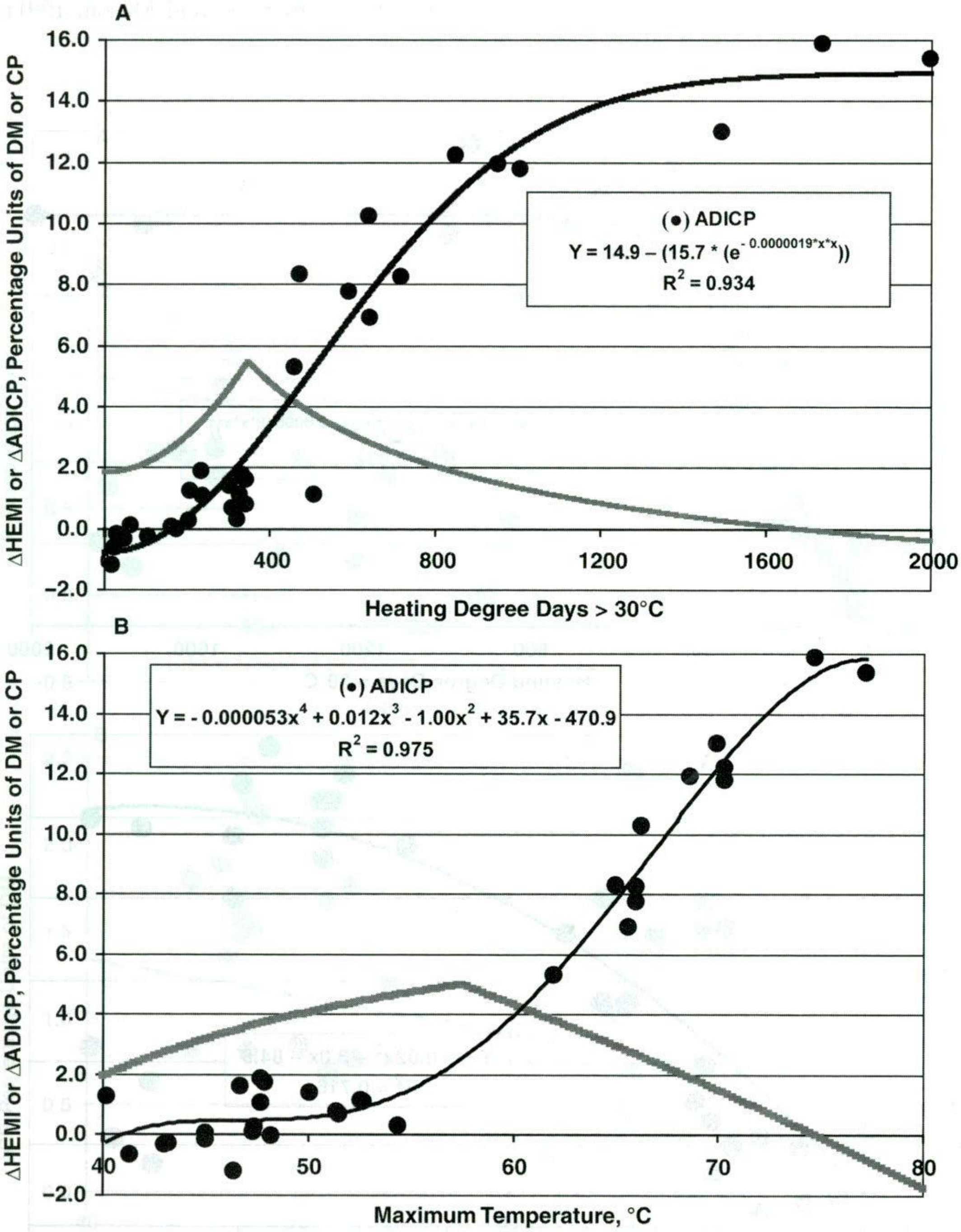

Figure 3. Regression curves illustrating the changes in concentrations of acid detergent insoluble CP (poststorage − prestorage; ΔADICP) and hemicellulose (ΔHEMI) as affected by A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage concentrations of ADICP and hemicellulose (weighted on the basis of the number of treatments from the low-moisture, intermediate-moisture, and high-moisture harvests) were 6.3% of CP and 15.1% of DM, respectively, which correspond generally to ΔADICP = 0 and ΔHEMI = 0 on the y-axis. Data for ΔADICP are represented by solid black circles for individual baling treatments and a solid black line for the regression equation. For ΔHEMI, regression curves are represented by solid gray lines. Regression equations for ΔHEMI have been described in detail previously (Coblentz and Hoffman, 2009b).

hemicellulose (approximated as NDF − ADF) within laboratory-scale or conventional rectangular bales of alfalfa that heated spontaneously (Coblentz et al., 1996) or within small 120-g lots of alfalfa hay that were heated by steam (100–130°C) or forced air (130–160°C; Yang et al., 1993).

Based on the results of the present study, there may be several reasons for these apparent discrepancies. Under conditions of modest spontaneous heating (<400 HDD or 55°C MAX), ΔADICP likely increased slowly via volatilization of ammonia from the hay (Rotz and Muck, 1994) and also by contributions of Maillard products that accumulated at a relatively slow rate. Over the same ranges of heating, changes in concentrations of hemicellulose as a result of storage (poststorage − prestorage; **ΔHEMI**) became increasingly positive in these same bales, reaching maxima at 347 HDD or 57.5°C MAX (Coblentz and Hoffman, 2009b). The mechanism for these responses was likely indirect, probably occurring because hemicellulose remained largely inert whereas nonstructural carbohydrates were the primary and preferential carbohydrates being oxidized. At greater HDD or MAX, ΔADICP increased at a much faster rate because the supply of nonstructural carbohydrates was largely exhausted and hemicellulose likely became a secondary reactive carbohydrate; as a result, ΔHEMI decreased sharply (Coblentz and Hoffman, 2009b). These diverse responses for ΔHEMI are superimposed over regressions of ΔADICP on HDD or MAX (Figures 3A and 3B); most notably, the inverse relationship between ΔADICP and ΔHEMI for large round bales that incurred the most severe spontaneous heating agrees closely with the early work of Goering et al. (1973). Although that early work continues to serve as the foundation for much of the theory explaining heat damage to forage proteins, our results suggest that their laboratory research model was consistent only with conditions of severe spontaneous heating in large hay packages that are likely unattainable if small rectangular bales stored in small stacks are used as the experimental unit or model.

In Situ Disappearance of CP

Kinetic parameters associated with ruminal in situ disappearance of CP from our spontaneously heated hays obtained from HM and IM harvests are summarized in Table 2. Data also include composite hays from the HM and IM harvests that were sampled before storage, and therefore represent unheated controls.

Fractions A, B, and C. Respective prestorage concentrations of fraction A from the HM and IM harvests were 42.7 and 39.5% of CP (Table 2), respectively, which agrees closely with an estimate for mid-maturity grass-legume hay with a majority percentage of legumes (42.4% of CP; NRC, 2001). Prestorage values for fraction B (44.4 and 47.5% of CP for HM and IM harvests, respectively) also were within proximity to NRC (2001) estimates (48.1% of CP), but observations for fraction C (12.9 and 13.0% of CP for HM and IM harvests, respectively) were 36% greater than published tabular values (NRC, 2001). Regressions of ΔA, ΔB, and ΔC on HDD all were curvilinear (Figure 4A), but the specific model for each fraction varied. The relationship between ΔA and HDD was best fitted to a quartic model exhibiting a very high coefficient of determination ($R^2 = 0.919$). In general, ΔA decreased with modest heating and was largely negative at <400 HDD. As heating became more extreme (>400 HDD), ΔA became positive, increasing sharply to a maximum of +12.4 percentage units of CP at 1,997 HDD. Generally, ΔB exhibited a mirror-opposite response that also was explained by a quartic model ($R^2 = 0.894$). This response became increasingly negative with severe heating, reaching a minimum of −14.1 percentage units of CP. Although ΔC was best fitted to a nonlinear regression model in which HDD was squared, the overall fit was poor ($R^2 = 0.337$). Generally, ΔC exhibited a relatively narrow range (−1.1 to 3.9 percentage units of CP) and remained positive for most hays (overall mean = 1.0 ± 1.13 percentage units of CP), thereby indicating that the pool of CP unavailable in the rumen generally increased with spontaneous heating, but these responses were challenging to define mathematically and relatively minor in scope compared with responses observed for ΔA and ΔB.

For regressions on MAX (Figure 4B), ΔA and ΔB again exhibited mirror-opposite responses. In each case, data were best fit to a quadratic model in which ΔA and ΔB became sharply positive and negative, respectively, particularly when MAX >60°C. For both response variables, the regression model again explained very high proportions of the variability within the data ($R^2 \geq 0.901$). For ΔC, the quartic regression model exhibited a substantially improved coefficient of determination ($R^2 = 0.612$) relative to the nonlinear model with HDD. In practical terms, this response did not differ substantially from that observed for HDD because it largely explained relatively minor oscillations for ΔC that remained slightly positive over most of our range for MAX. Taken in total, these responses suggest that most of the changes in partitioning of CP among various pools based on susceptibility to ruminal degradation occurred between fractions A and B, and that fraction C was affected minimally by spontaneous heating.

Previous research measuring shifts in CP fractions in response to heating, or conservation as hay, or both have yielded inconsistent results. Using an inhibitor in

Table 2. In situ disappearance kinetics of CP for 20 baling treatments selected from the high- and intermediate-moisture harvests[1]

Item[2]	Bale diameter, m	Initial bale moisture, %	HDD, d	MAX, °C	Fraction, % of CP A	B	C	Lag time, h	K_d, /h	RDP,[3] % of CP
High-moisture harvest										
1	Prestorage composite[4]		0	—	42.7	44.4	12.9	1.84	0.167	75.1
2	0.9	26.7	321	54.4	44.3	42.3	13.4	1.91	0.094	69.7
3	0.9	38.7	470	65.0	45.0	38.2	16.8	1.76	0.094	68.2
4	0.9	41.9	590	66.0	45.8	40.3	13.9	2.27	0.087	69.4
5	1.2	30.9	641	65.6	45.4	39.5	15.1	2.58	0.074	67.2
6	1.5	32.1	716	66.0	45.3	40.0	14.7	1.83	0.076	67.5
7	1.2	39.4	952	68.6	48.6	36.9	14.5	2.34	0.071	68.6
8	1.2	43.5	1,005	70.3	53.1	33.7	13.2	2.90	0.085	72.8
9	1.5	38.7	1,494	69.9	49.4	37.2	13.4	2.93	0.095	71.9
10	1.5	40.1	1,737	74.7	51.3	34.7	14.0	3.87	0.102	72.9
11	1.5	46.6	1,997	77.2	55.1	30.3	14.6	2.36	0.083	72.6
SEM	—	—	—	—	0.40	0.53	0.36	0.479	0.0088	0.73
Intermediate-moisture harvest										
12	Prestorage composite[5]		0	—	39.5	47.5	13.0	1.18	0.173	74.6
13	0.9	17.1	29	45.0	40.5	46.1	13.4	2.07	0.176	74.9
14	1.2	17.5	62	47.3	39.1	48.9	12.0	1.73	0.161	74.2
15	1.2	18.9	175	48.2	38.4	48.1	13.5	1.94	0.144	72.3
16	1.5	16.8	203	47.4	37.9	49.4	12.7	1.18	0.126	71.4
17	1.5	22.0	304	50.1	38.5	48.8	12.7	1.12	0.120	70.8
18	1.5	20.3	308	51.5	38.1	47.3	14.6	1.83	0.111	68.7
19	1.5	24.2	326	52.7	39.2	46.2	14.5	1.82	0.122	70.0
20	1.2	22.8	506	52.6	40.0	46.2	13.7	1.62	0.107	69.3
SEM	—	—	—	—	0.24	0.40	0.31	0.38	0.0122	0.84

[1]HDD = heating degree days >30°C that were accumulated during bale storage. MAX = maximum internal bale temperature. Fractions: A = fraction of total CP pool disappearing at a rate too rapid to measure; B = fraction of total CP pool disappearing at a measurable rate; C = fraction of total CP pool unavailable in the rumen. K_d = fractional rate constant.

[2]Numbers are assigned arbitrarily and denote individual haying treatments evaluated by in situ methods.

[3]Calculated as $A + B \times [(K_d + Kp)/K_d]$, where Kp was the ruminal passage rate, which was arbitrarily set at 0.06/h (Hoffman et al., 1993).

[4]Composite equally weighted with sample obtained immediately after baling from hays 2 through 11.

[5]Composite equally weighted with sample obtained immediately after baling from hays 13 through 20.

vitro technique, Broderick et al. (1992) observed small increases (2.4 percentage units of CP) for fraction A in alfalfa hays as compared with lyophilized fresh forage. Conversely, fraction B decreased with conservation as hay by a similar amount. Using similar methodologies, Yang et al. (1993) observed decreases for both fraction A and B within alfalfa hays heated externally (forced air or steam) relative to unheated control hays. For hays that have heated spontaneously, Coblentz et al. (1997) reported greater concentrations of fraction A in alfalfa bales sampled before storage compared with those incurring minimal spontaneous heating; however, when prestorage hays were not considered, fraction A increased linearly with HDD at a rate of 0.027 percentage units of CP per HDD. Inverse relationships were observed for fraction B, which agrees with our current work (Figures 4A and 4B). Finally, McBeth et al. (2003) reported that fraction B did not differ among bermudagrass hays packaged in small rectangular bales and incurring 5 to 401 HDD during storage. Although fraction A differed among those same hays, responses were confined to relatively small oscillations within a narrow range that were of questionable biological relevance. Although our diverging responses for ΔA and ΔB are consistent with those reported by Coblentz et al. (1997), there are a couple of obvious differences. First, our continuum of heating (either HDD or MAX) extended well beyond that reported previously. Second, ΔA reached a maximum of +12.4 percentage units of CP in the most severely heated hays, which is a far greater change than reported in other studies.

Lag Time. Lag times determined from the ruminal nonlinear decay model increased with spontaneous heating but varied erratically (0.57 ± 0.521 h) with HDD or MAX (data not shown). The regression of ΔLAG on HDD was best fit to a cubic model that explained approximately half of the variability within the data ($Y = -0.0000000018x^3 + 0.0000054x^2 - 0.0037x + 0.93$; $R^2 = 0.570$). In contrast, ΔLAG could not be related to MAX by quartic, cubic, quadratic, or linear models ($P \geq 0.059$).

K_d. Estimates of K_d determined on a prestorage basis were 0.167 and 0.173/h for the HM and IM harvests, respectively (Table 2). These estimates are slightly greater than reported for mid-maturity legume-grass mixtures comprised predominantly of legume forage

(0.155/h; NRC, 2001) but somewhat slower than reports for early- to mid-bloom alfalfa (0.213/h, Coblentz et al., 1998; 0.210/h, Hoffman et al., 1993). Regressions of ΔK_d on HDD (Figure 5A) and MAX (Figure 5B) both were best fit by cubic models in which ΔK_d became sharply negative while HDD <1,000 and MAX <60°C, thereby indicating a reduced degradation rate for CP. At more intense heating increments, ΔK_d either stabilized or increased slightly. The erratic observations of ΔK_d at the most extreme levels of heating precluded

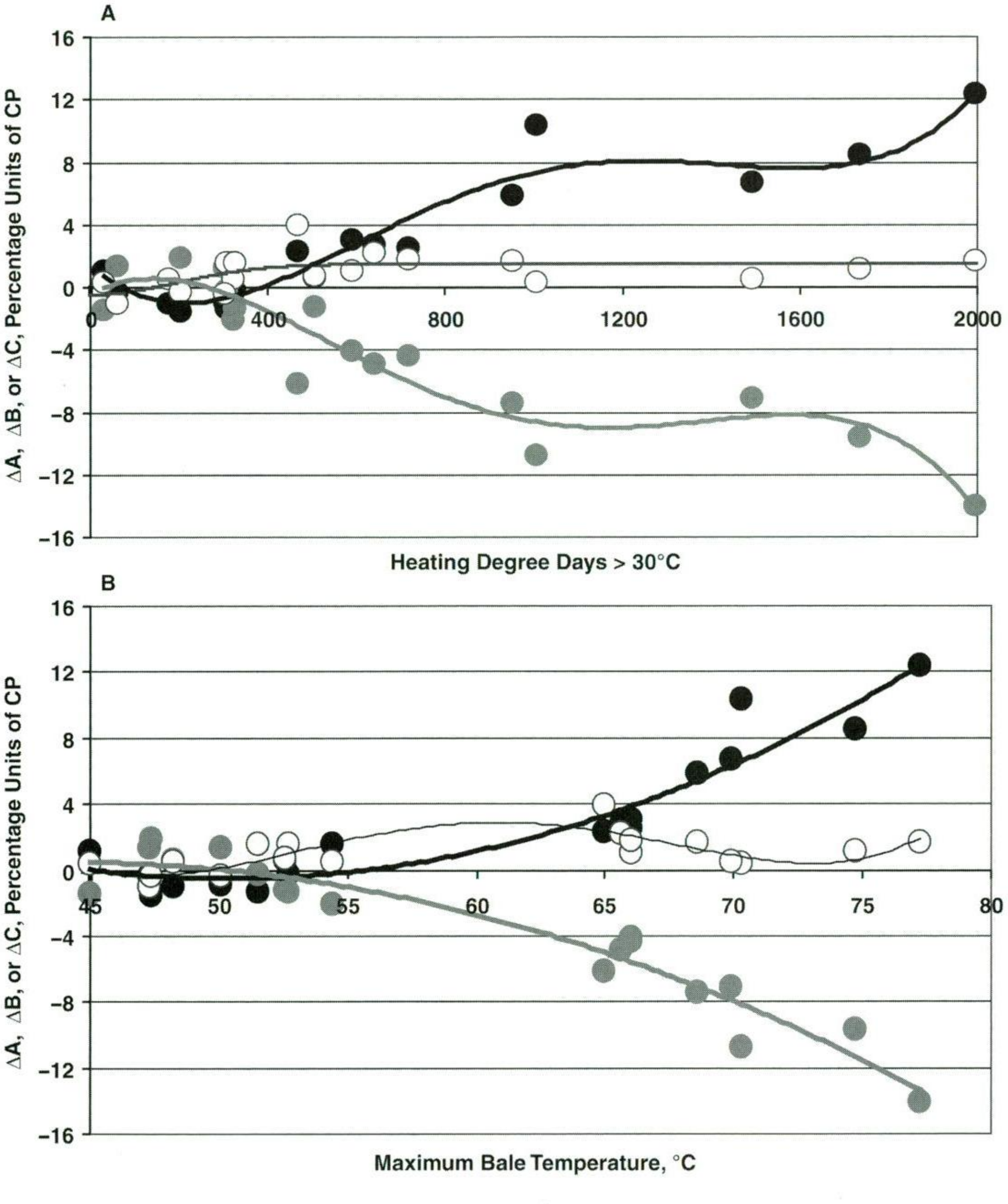

Figure 4. Regressions illustrating the changes (poststorage − prestorage) for percentage of CP disappearing from Dacron bags at a rate too rapid to measure (ΔA; solid black circles, thick black line), at a measureable rate (ΔB; solid gray circles, thick gray line), and unavailable in the rumen (ΔC; open circles, thin black line) from alfalfa-orchardgrass hays as affected by A) heating degree days >30°C and B) maximum internal bale temperature. Mean prestorage concentrations of fractions A, B, and C (weighted on the basis of the number of treatments from the intermediate-moisture and high-moisture harvests) were 41.3, 45.8, and 12.9% of CP, respectively, which correspond generally to ΔA, ΔB, and $\Delta C = 0$ on the y-axis. For panel A, regression equations are defined as follows. ΔA: $Y = 0.000000000014x^4 - 0.000000058x^3 + 0.000074x^2 - 0.024x + 1.4$, $R^2 = 0.919$; ΔB: $Y = -0.000000000014x^4 + 0.000000055x^3 - 0.000064x^2 + 0.015x - 0.4$, $R^2 = 0.894$; ΔC, $Y = 1.97 \times (e^{-0.000013 \times x \times x \times x}) - 1.45$, $R^2 = 0.337$. For panel B, equations are defined as follows. ΔA: $Y = 0.018x^2 - 1.80x + 44.9$, $R^2 = 0.901$; ΔB: $Y = -0.012x^2 + 1.10x - 23.6$, $R^2 = 0.923$; ΔC: $Y = 0.00010x^4 - 0.025x^3 + 2.24x^2 - 87.6x + 1{,}263.1$, $R^2 = 0.612$.

selection of a nonlinear decay model that establishes an asymptote, thereby differing from observations associated with disappearance kinetics of DM (Coblentz and Hoffman, 2009a) and NDF (Coblentz and Hoffman, 2009b) for these hays. However, ΔK_d for ruminal disappearance of CP was related closely to both HDD ($R^2 = 0.939$) and MAX ($R^2 = 0.876$), which was consistent with the previously defined relationships for disappearance rates of DM and NDF from these hays. It is not clear whether the relatively small and erratic increases in ΔK_d when HDD >1,000 or MAX >60°C that resulted in optimum fits for cubic regression models reflect biologically meaningful changes in disappearance kinetics of CP or whether these unexpected responses simply reflect the imprecise nature of the measurement and mask what is effectively an asymptotic response for ΔK_d within severely heated bales.

Previously, K_d has been shown to decrease linearly with HDD in alfalfa hays ($Y = -0.00017 + 0.139$; $r^2 = 0.856$; Coblentz et al., 1997). Although the relationship between ΔK_d and HDD was curvilinear in the present study (Figure 5A), arbitrarily confining our data to <400 HDD yields a regression that is likewise linear ($P < 0.001$; $n = 8$; $Y = -0.00021x + 0.004$; $r^2 = 0.904$) with a slope that is similar to that reported previously. Restricting HDD to <400 establishes a range that is comparable to that observed for conventional (approximately 45-kg) rectangular bales of alfalfa hay stored in small stacks (Coblentz et al., 1996). The relationship between ΔK_d and HDD begins to lose its strictly linear character when bales that accumulated between 400 and 1,000 HDD are included in the regression model ($n = 14$; $Y = 0.00000013x^2 - 0.00023x + 0.003$; $R^2 = 0.937$); this degree of heating is still easily attainable under field conditions for large round or large rectangular hay bales. Slight increases in ΔK_d were observed only in the most severely heated bales (HDD >1,000) that would likely result only under the most extreme conditions of (poorly managed) commercial hay production. For MAX, a close linear relationship ($n = 9$; $Y = -0.0074 + 0.326$; $r^2 = 0.804$) between ΔK_d and MAX was observed when MAX was confined arbitrarily to <60°C. In total, these data suggest that within the range of heating most relevant to production situations, the inverse relationship between K_d and measures of spontaneous heating is primarily linear. Furthermore, the consequences of this relationship are practically relevant; relative to prestorage estimates, the heating increment required to reduce K_d by 50% occurred at about 500 HDD or 60°C MAX, both of which are easily attainable under field conditions.

RDP. Prestorage estimates of RDP from the HM and IM harvests were 75.1 and 74.6% of CP, respectively (Table 2), which is slightly less than the NRC (2001) estimate (79.0% of CP) for this forage type. The ΔRDP of poststorage hays decreased sharply (Figure 6A) with modest heating, reaching a minimum of −7.9 percentage units of CP. However, ΔRDP increased thereafter to the extent that the mean ΔRDP of the 4 most severely heated hays was about −2.6 percentage units of CP. This response was best fit to a cubic model with a relatively high coefficient of determination ($R^2 = 0.802$). In contrast, the regression of ΔRDP on MAX (Figure 6B) was best fitted to a quadratic model, although the coefficient of determination was slightly reduced ($R^2 = 0.734$).

In practice, regressions of ΔRDP on HDD and MAX exhibited many characteristics similar to those observed for ΔK_d (Figures 6A and 6B). The most important of these is the strict linear nature of the relationship when HDD <400 ($n = 8$; $Y = -0.018x + 0.7$; $r^2 = 0.925$) and when MAX <60°C ($n = 9$; $Y = -0.63x + 28.2$; $r^2 = 0.806$). For HDD, the slope within this limited range is identical to that reported previously for alfalfa hay packaged in laboratory-scale or conventional rectangular packages (−0.018 percentage units of CP/HDD; Coblentz et al., 1997). The overall regression relationship for ΔRDP on HDD began to exhibit significant quadratic character when the pool of baling treatments was widened to include those with <1,000 HDD ($n = 14$; $Y = 0.000015x^2 - 0.022x + 0.8$; $R^2 = 0.913$). For the 4 most severely heated hays (HDD >1,000), ΔRDP increased substantially, largely because of concurrent increases in ΔA. Fraction A is assumed generally to be immediately available in the rumen; however, it also can include physical leakage of rumen-undegradable CP from Dacron bags (Broderick, 1994). For severely heated hays in this study, it remains unclear whether the observed increases in ΔRDP are relevant in vivo or whether the severity of the treatments simply exceeds the reliable limits of the in situ method.

In Situ Disappearance of NDICP

Fractions A_{ND}, B_{ND}, and C_{ND}. On a prestorage basis, fraction A_{ND} made up 0.4 and 0.0% of the total pool of NDICP (Table 3) for the HM and IM harvests, respectively. These values are consistent with those made previously for leaf, stem, or whole-plant alfalfa (Coblentz et al., 1999; Ogden et al., 2006). Theoretically, NDF is insoluble in water (Van Soest, 1982); therefore, CP associated with this NDF should be fully recoverable following machine rinsing of 0-h in situ bags. In reality, NDF often is incompletely recovered following machine rinsing during in situ evaluations, especially when the forage under consideration is comprised of relatively immature cool-season grasses (Hoffman et al., 1993; Flores et al., 2007; Coblentz and Hoffman, 2009b).

Although the HM and IM harvests contained 31 and 22% vegetative orchardgrass, respectively, and fraction A associated with ruminal disappearance of NDF for these hays made up 14.0 and 16.0% of the total NDF pool (Coblentz and Hoffman. 2009b), our data suggest that the CP associated with this NDF pool was fully recovered after machine rinsing of 0-h bags.

Before storage, fraction B_{ND} made up 88.8 and 87.6% of NDICP for the HM and IM harvests, respectively. These percentages of the NDICP pool were considerably greater than those reported in other studies for alfalfa (65.7%, Coblentz et al., 1999; 59.0%, Ogden et al., 2006) but were similar to an estimate for vegetative orchardgrass hay (88.0%, Ogden et al., 2006). Fraction C_{ND}, which represents CP bound within the NDF matrix that is not available within the rumen, made up 10.8 and 12.4% of the NDICP pool for HM and IM harvests, respectively. As observed for fraction

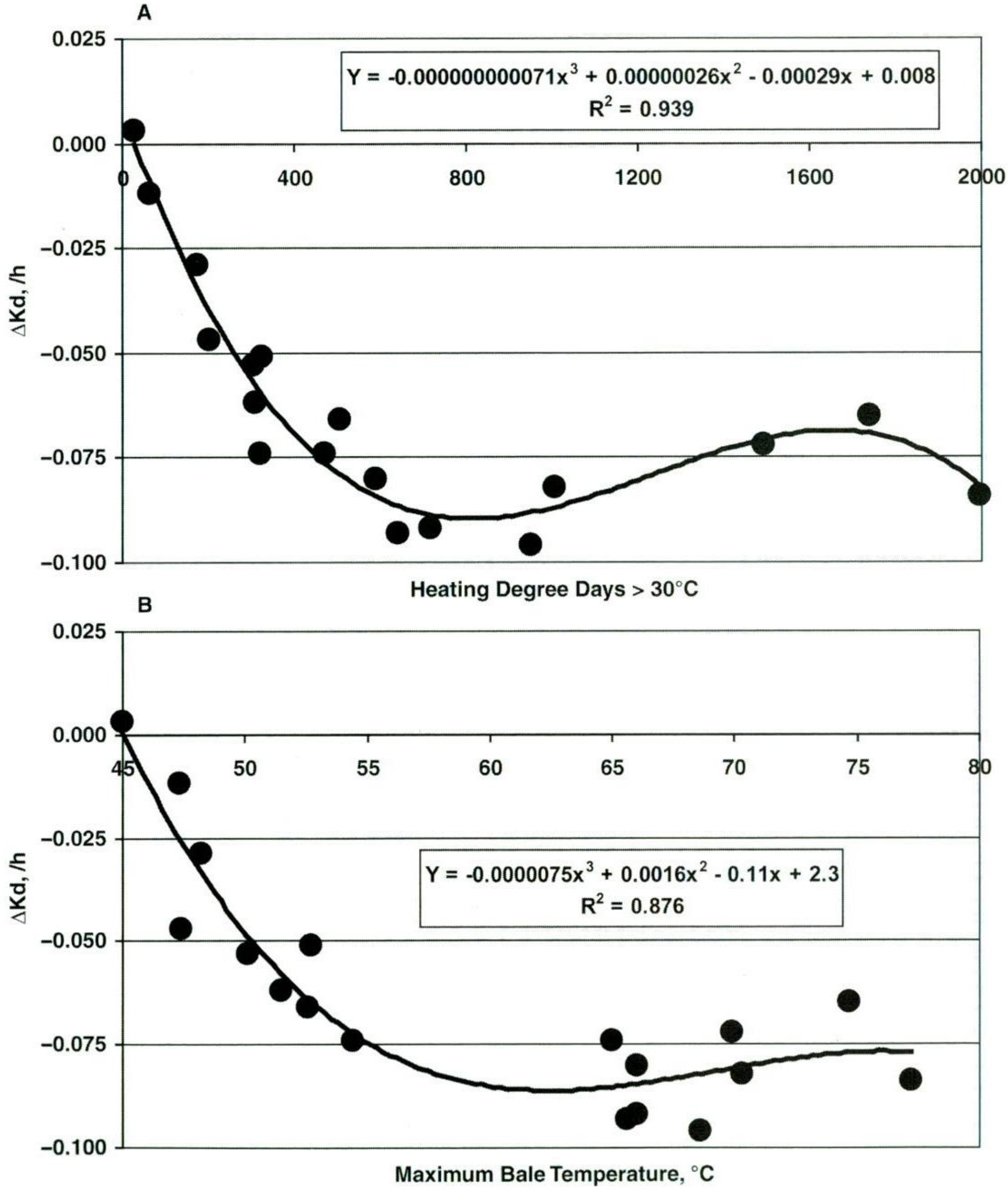

Figure 5. Regressions of the changes for ruminal in situ disappearance rate of CP (poststorage − prestorage; ΔK_d) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage K_d (weighted on the basis of the number of treatments selected from the intermediate-moisture and high-moisture harvests) was 0.169/h, which corresponds generally to $\Delta K_d = 0$ on the y-axis.

B_{ND}, these percentages of the total NDICP pool more closely match those reported previously for vegetative orchardgrass hay (9.3%; Ogden et al., 2006) than for alfalfa forages (34.2%, Coblentz et al., 1999; 41.0%; Ogden et al., 2006).

As a consequence of spontaneous heating, ΔA_{ND} increased linearly ($r^2 = 0.778$) with HDD (Figure 7A), reaching a maximum of +23.8 percentage units of NDICP in the most severely heated hay (1,997 HDD). For the regression on MAX (Figure 7B), the data were best fitted to a quadratic regression model that exhibited a coefficient of determination ($R^2 = 0.764$) similar to that observed for HDD. In contrast, ΔB_{ND} generally decreased as HDD or MAX increased and became most negative in severely heated hays, reaching a minimum of −22.3 percentage units of NDICP. Regression relationships between ΔB_{ND} and HDD or MAX were curvilinear in both cases. For HDD, data were best fitted to a quartic model ($R^2 = 0.840$), whereas the regression on MAX exhibited a quadratic response ($R^2 = 0.795$).

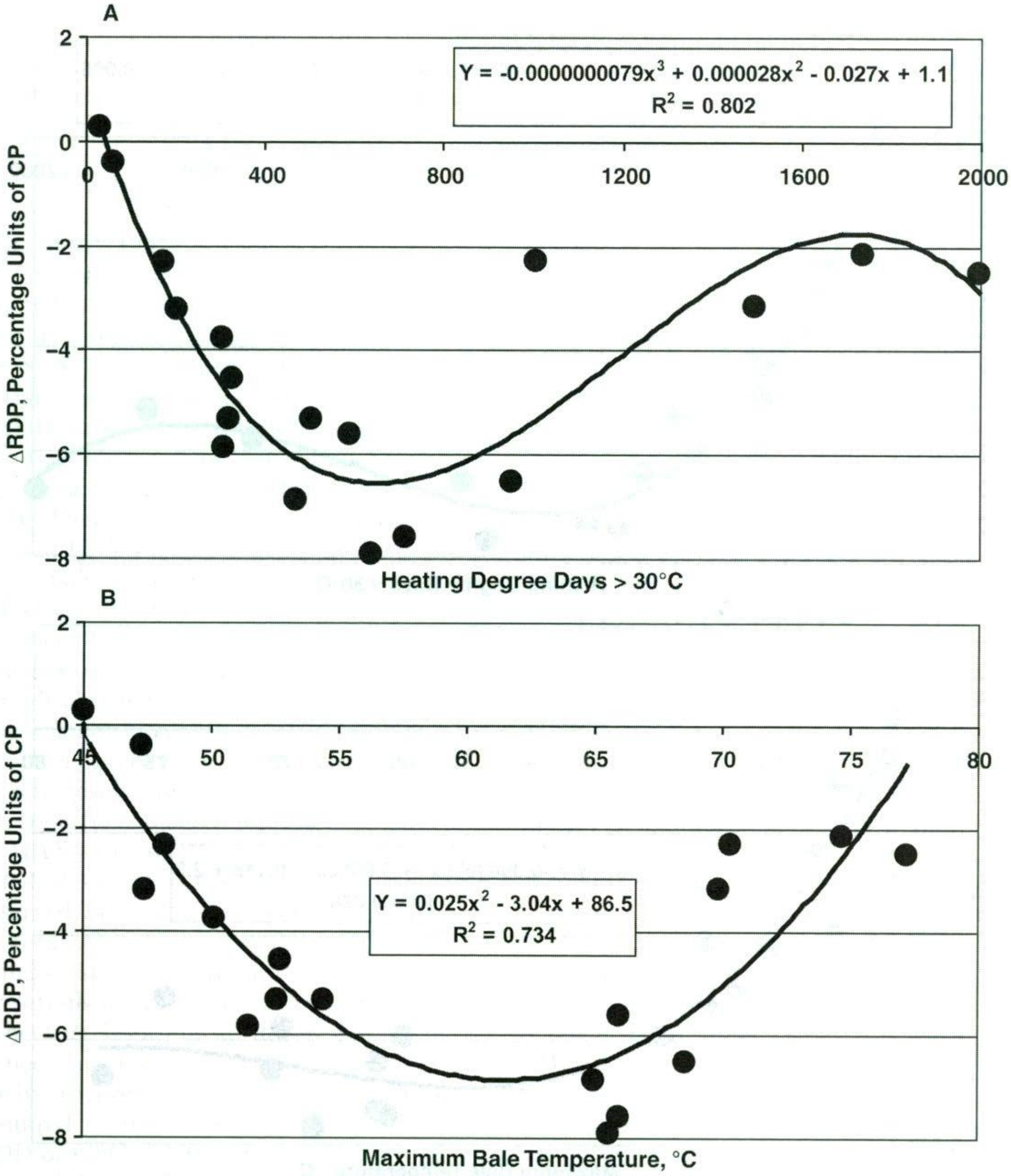

Figure 6. Regressions of the changes in effective ruminal disappearance of CP (poststorage − prestorage; ΔRDP) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean RDP of CP (weighted on the basis of the number of treatments from the intermediate-moisture and high-moisture harvests) was 74.8% of CP, which corresponds generally to ΔRDP = 0 on the y-axis. Effective degradability was calculated on the basis of a ruminal passage rate of 0.060/h.

Table 3. In situ disappearance kinetics of neutral detergent insoluble CP (NDICP) for 20 baling treatments selected from the high- and intermediate-moisture harvests[1]

					Fraction, % of NDICP					RDP_{ND}[3]	
Item[2]	Bale diameter, m	Initial bale moisture, %	HDD, d	MAX, °C	A_{ND}	B_{ND}	C_{ND}	Lag time, h	K_{dND}, /h	% of NDICP[4]	% of CP[5]
High-moisture harvest											
1	Prestorage composite[6]		0	—	0.4	88.8	10.8	3.01	0.230	70.7	13.0
2	0.9	26.7	321	54.4	5.8	87.2	7.0	1.89	0.153	68.3	25.4
3	0.9	38.7	470	65.0	3.9	85.6	10.6	2.90	0.160	65.6	30.7
4	0.9	41.9	590	66.0	5.3	86.4	8.4	2.52	0.140	65.2	27.1
5	1.2	30.9	641	65.6	3.5	88.0	8.5	2.66	0.157	67.0	30.4
6	1.5	32.1	716	66.0	3.1	87.5	9.4	1.55	0.123	61.5	26.9
7	1.2	39.4	952	68.6	15.2	75.6	9.2	2.34	0.124	65.3	31.7
8	1.2	43.5	1,005	70.3	11.1	79.4	9.5	2.53	0.146	66.8	28.8
9	1.5	38.7	1,494	69.9	14.4	76.4	9.2	2.12	0.130	66.5	30.8
10	1.5	40.1	1,737	74.7	10.2	81.5	8.4	1.49	0.127	65.3	28.6
11	1.5	46.6	1,997	77.2	24.2	66.5	9.3	2.94	0.110	67.1	29.7
SEM	—	—	—	—	0.45	0.70	0.43	0.438	0.0130	1.36	0.61
Intermediate-moisture harvest											
12	Prestorage composite[7]		0	—	0.0	87.6	12.4	2.27	0.234	69.4	15.2
13	0.9	17.1	29	45.0	0.0	88.3	11.7	2.64	0.205	67.9	15.5
14	1.2	17.5	62	47.3	0.0	91.0	9.0	2.32	0.195	69.2	17.3
15	1.2	18.9	175	48.2	1.2	88.8	10.0	3.41	0.191	68.1	20.2
16	1.5	16.8	203	47.4	0.1	90.9	9.0	2.65	0.204	70.1	19.8
17	1.5	22.0	304	50.1	4.2	87.1	8.7	2.58	0.204	71.2	28.2
18	1.5	20.3	308	51.5	0.0	92.0	8.0	2.24	0.160	66.8	23.7
19	1.5	24.2	326	52.7	0.0	91.4	8.6	2.35	0.173	67.2	23.9
20	1.2	22.8	506	52.6	0.0	92.6	7.4	2.18	0.175	67.5	28.3
SEM	—	—	—	—	0.54	0.67	0.35	0.368	0.0220	1.82	0.62

[1]HDD = heating degree days >30°C that were accumulated during bale storage. MAX = maximum internal bale temperature. Fractions: A_{ND} = fraction of total NDICP pool disappearing at a rate too rapid to measure; B_{ND} = fraction of total NDICP pool disappearing at a measurable rate; C_{ND} = fraction of total NDICP pool unavailable in the rumen. K_{dND} = fractional rate constant for NDICP. RDP_{ND} = rumen-degradable neutral detergent insoluble CP.

[2]Numbers are assigned arbitrarily and denote individual haying treatments evaluated by in situ methods.

[3]RDP_{ND} calculated as $A_{ND} + B_{ND} \times [(K_{dND} + Kp)/K_{dND}]$, where Kp was the ruminal passage rate, which was arbitrarily set at 0.06/h (Hoffman et al., 1993).

[4]RDP_{ND} expressed as a percentage of NDICP.

[5]RDP_{ND} expressed as a percentage of the entire pool of CP within the hay.

[6]Composite equally weighted with sample obtained immediately after baling (prestorage) from hays 2 through 11.

[7]Composite equally weighted with sample obtained immediately after baling (prestorage) from hays 13 through 20.

McBeth et al. (2003) also reported an inverse relationship between fraction B_{ND} and HDD for bermudagrass hays packaged in small rectangular bales, although the maximum accumulation of 401 HDD was less than observed in the present study. Over the entire ranges of HDD and MAX, ΔC_{ND} remained negative (overall mean = −2.5 ± 1.35 percentage units of NDICP), indicating the percentage of the total NDICP pool that was unavailable in the rumen decreased minimally with spontaneous heating. Regressions of ΔC_{ND} on HDD and MAX both were fitted to complex quartic models, but the biological relevance of their complex nature is questionable given the limited magnitude and narrow range of the estimates.

Lag Time. Regressions of ΔLAG_{ND} on HDD and MAX were both curvilinear (data not shown); for HDD, the best model was quadratic ($Y = 0.00000095x^2 - 0.0025x + 0.65$; $R^2 = 0.478$), whereas a nonlinear model explained the greatest proportion of the response for the regression on MAX ($Y = 11.7 \times (e^{-0.00103 \times x \times x}) - 0.79$; $R^2 = 0.503$). Generally, ΔLAG_{ND} was mostly positive for baling treatments incurring <400 HDD or <55°C MAX but then decreased and remained negative at all greater increments of spontaneous heating.

K_{dND}. Prestorage estimates of K_{dND} were 0.230 and 0.234/h for HM and IM harvests, respectively (Table 3), which are modestly faster than 2 previous determinations for alfalfa forages (0.138/h, Coblentz et al., 1999; 0.150/h, Ogden et al., 2006). Regressions of ΔK_{dND} on both HDD and MAX were best fitted to nonlinear decay models (Figures 8A and 8B) in which residuals were minimized without squaring the independent variable. For regressions on both HDD and MAX, ΔK_{dND} was closely associated with spontaneous heating, exhibiting respective R^2 statistics of 0.792 and 0.833. Within these regression relationships, ΔK_{dND} became asymptotic at

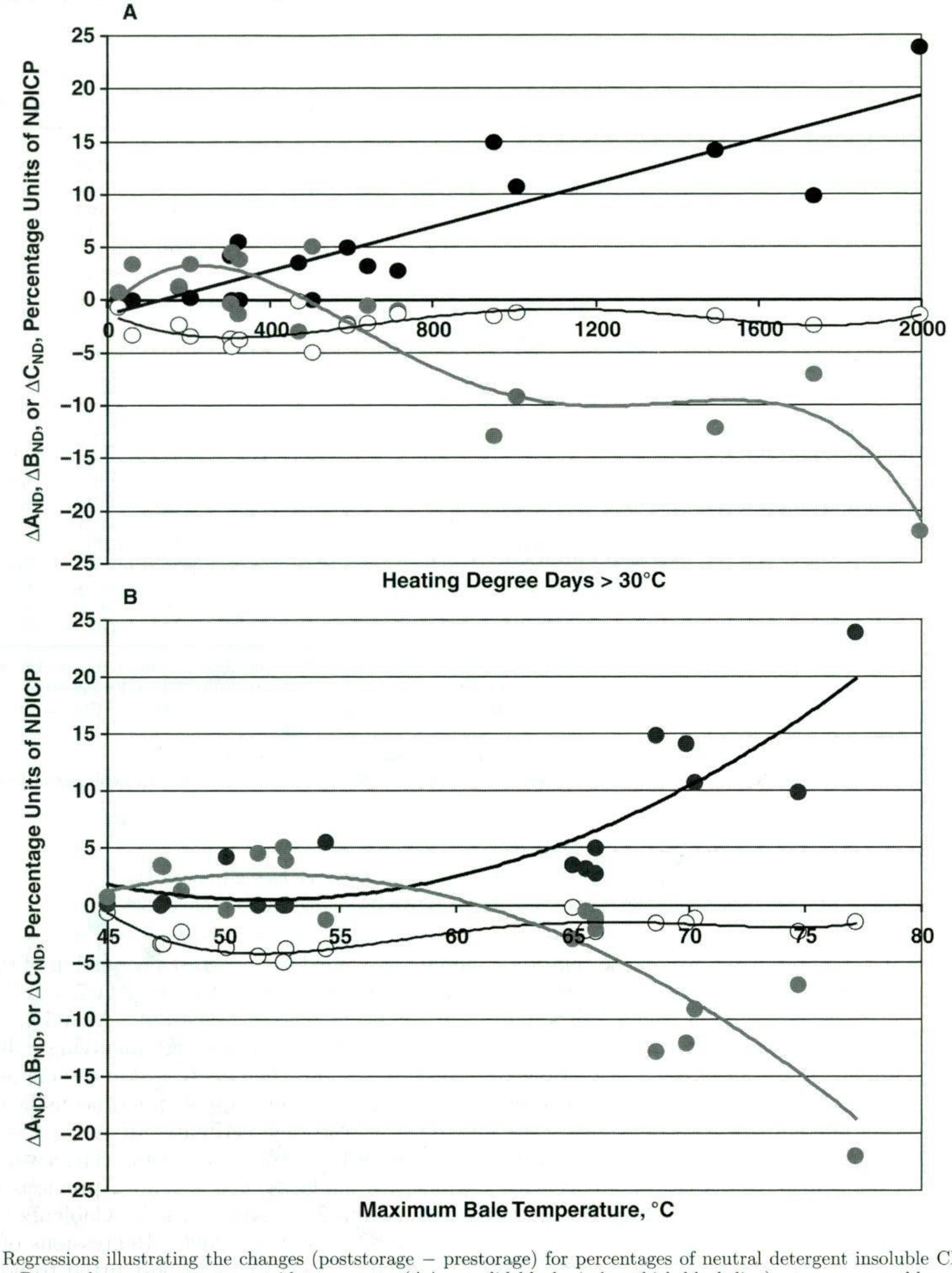

Figure 7. Regressions illustrating the changes (poststorage − prestorage) for percentages of neutral detergent insoluble CP (NDICP) disappearing from Dacron bags at a rate too rapid to measure (ΔA_{ND}; solid black circles, thick black line), at a measureable rate (ΔB_{ND}; solid gray circles, thick gray line), and unavailable in the rumen (ΔC_{ND}; open circles, thin black line) from alfalfa-orchardgrass hays as affected by A) heating degree days >30°C and B) maximum internal bale temperature. Mean prestorage concentrations of fractions A_{ND}, B_{ND}, and C_{ND} (weighted on the basis of the number of treatments from the intermediate-moisture and high-moisture harvests) were 0.2, 88.3, and 11.5% of NDICP, respectively, which correspond generally to ΔA_{ND}, ΔB_{ND}, and $\Delta C_{ND} = 0$ on the y-axis. For figure A, regression equations are defined as follows. ΔA_{ND}: $Y = 0.010x - 1.3$, $R^2 = 0.778$; ΔB_{ND}: $Y = -0.000000000025x^4 + 0.00000010x^3 - 0.00012x^2 + 0.042x - 0.9$, $R^2 = 0.840$; ΔC_{ND}: $Y = 0.0000000000073x^4 - 0.000000031x^3 + 0.000041x^2 - 0.017x - 1.2$, $R^2 = 0.421$. For figure B, equations are defined as follows. ΔA_{ND}: $Y = 0.030x^2 - 3.08x + 80.3$, $R^2 = 0.764$; ΔB_{ND}: $Y = -0.033x^2 + 3.44x - 86.3$, $R^2 = 0.795$; ΔC_{ND}: $Y = 0.000079x^4 - 0.020x^3 + 1.93x^2 - 80.6x + 1{,}243.1$, $R^2 = 0.800$.

−0.117 and −0.138/h for HDD and MAX, respectively. This represents at least a 50% reduction for K_{dND} relative to hays sampled on a prestorage basis. However, even in hays incurring minimal spontaneous heating, ΔK_{dND} was reduced by −0.029/h, which is a reduction of about 13% relative to prestorage controls and indicates that K_{dND} is reduced during storage, even without meaningful heating. In a previous report, K_{dND} decreased from 0.056 to 0.036/h for bermudagrass hays incurring between 5 and 401 HDD during storage (McBeth et al., 2003), which is consistent with our observations.

***RDP*$_{ND}$.** Calculated at a 0.06/h passage rate, our estimates of RDP_{ND} for prestorage controls were 70.7 and 69.4% of NDICP for the HM and IM harvests,

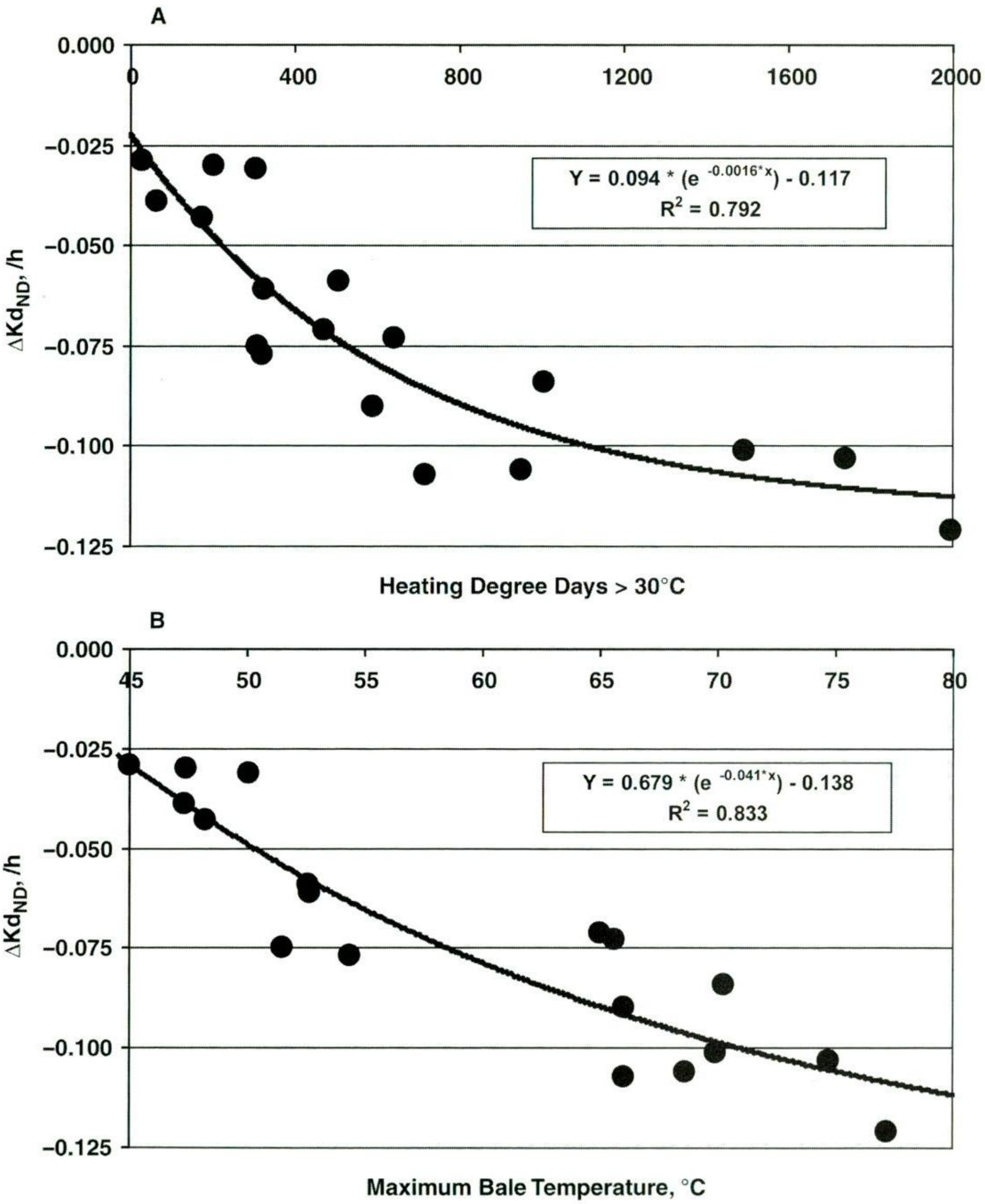

Figure 8. Regressions of the changes for ruminal in situ disappearance rate of neutral detergent insoluble CP (NDICP) (poststorage − prestorage; ΔK_{dND}) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage K_{dND} (weighted on the basis of the number of treatments selected from the intermediate-moisture and high-moisture harvests) was 0.232/h, which corresponds generally to $\Delta K_{dND} = 0$ on the y-axis.

respectively. These estimates indicate more extensive ruminal disappearance than has been reported previously for alfalfa forages (43.5% of NDICP, Coblentz et al., 1999; 50.5% of NDICP, Ogden et al., 2006), but the observations are consistent with a previous estimate for vegetative orchardgrass hay (72.1% of NDICP, Ogden et al., 2006). Regressions of ΔRDP_{ND} on HDD (Figure 9A) and MAX (Figure 9B) described a decreasing trend for ΔRDP_{ND} as heating increments within bales became greater, which is consistent with observed responses in other work (McBeth et al., 2003). Data were best fitted to a quadratic model for HDD and a nonlinear decay model for MAX that required squaring of the independent variable. In the latter case, ΔRDP_{ND} became asymptotic at −5.8 percentage units of NDICP. Unlike the regressions of ΔK_{dND} with HDD or MAX, the distribution of data points was somewhat erratic, resulting in greater residuals and depressed coefficients

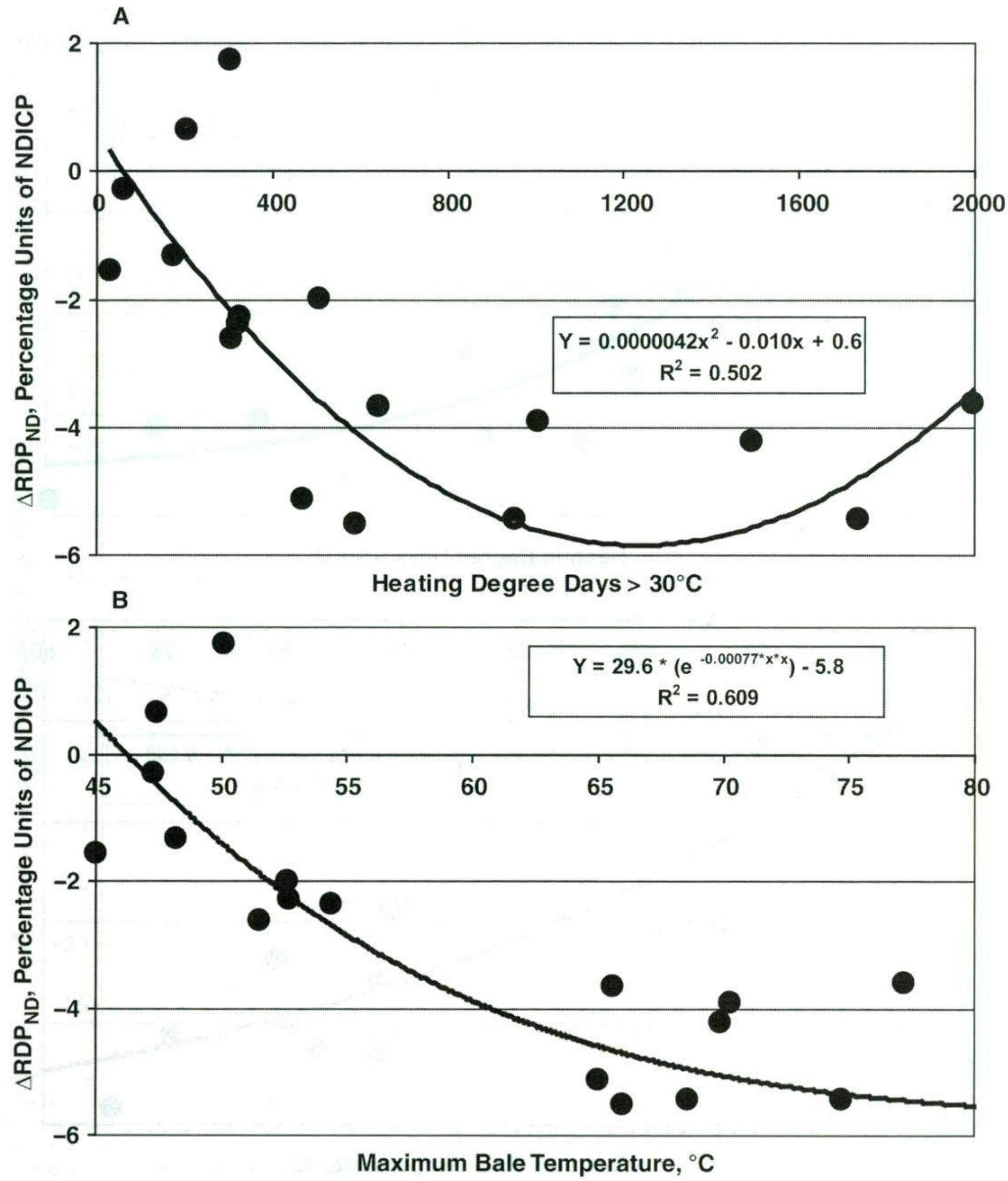

Figure 9. Regressions of the changes in effective ruminal disappearance of neutral detergent insoluble CP (NDICP) (poststorage − prestorage; ΔRDP_{ND}) on A) heating degree days >30°C and B) maximum internal bale temperature. The mean prestorage RDP_{ND} of NDICP (weighted on the basis of the number of treatments from the intermediate-moisture and high-moisture harvests) was 70.1% of NDICP, which corresponds generally to $\Delta RDP_{ND} = 0$ on the y-axis. Effective degradability was calculated on the basis of a ruminal passage rate of 0.060/h.

of determination for both independent variables ($R^2 \geq 0.502$).

The curvilinear trends that describe the inverse relationship between ΔRDP_{ND} and HDD or MAX are somewhat complex and mask conflicting responses to spontaneous heating among various NDICP pools. The relatively close regression relationships between ΔK_{dND} and measures of spontaneous heating (Figures 8A and 8B) suggest that the ruminal availability of fraction B_{ND} is altered negatively by spontaneous heating. However, these responses were offset by a concomitant expansion of the total pool of NDICP with heating (Figure 2A and 2B); within this expanded NDICP pool, an increasing percentage comprised fraction A_{ND} (Figures 7A and 7B), which is assumed to be immediately available in the rumen.

Contributions to RDP Based on Solubility in Neutral Detergent

Estimates of ΔRDP were negative across the entire range of HDD (Figure 6A) or MAX (Figure 6B) for our baling treatments, indicating that overall ruminal disappearance of forage CP was suppressed by spontaneous heating; however, these respective cubic and quadratic responses obscure rather complex and dynamic changes within various pools of CP. To illustrate this concept, degradation of the total forage CP pool was subdivided on the basis of solubility in neutral detergent (Figure 10). On this basis, ΔRDP_{ND} increased with HDD in a pattern best explained with a nonlinear model in which HDD were squared. This relationship became asymptotic at +16.4 percentage units of CP when approximately 750 HDD were accumulated. The asymptotic nature of this regression model is unique because it is made up of sharply conflicting responses for ΔA_{ND} and ΔB_{ND} pools (Figures 7A and 7B) that have been discussed previously. Despite these fluxes among NDICP pools, the nonlinear regression model was very effective at describing the overall relationship between ΔRDP_{ND} and HDD ($R^2 = 0.902$). In contrast to the response for ΔRDP_{ND}, changes (poststorage − prestorage) in contributions from neutral detergent soluble CP (**ΔRDP_{NDS}**) became sharply negative with spontane-

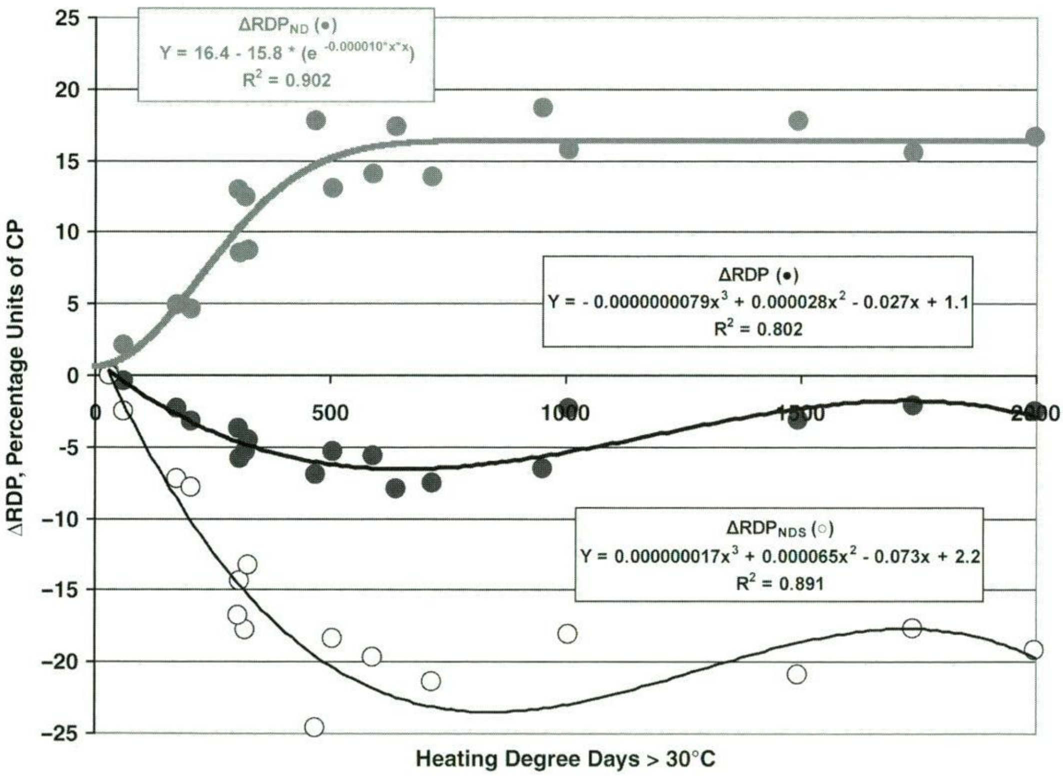

Figure 10. Regressions of the changes in RDP (poststorage − prestorage; ΔRDP, solid black circles, thick black line) on heating degree days >30°C, as well as changing contributions to the cumulative pool of RDP from rumen-degradable neutral detergent insoluble CP (NDICP) (ΔRDP_{ND}, solid gray circles, thick gray line) and rumen-degradable neutral detergent soluble CP (ΔRDP_{NDS}, open circles, thin black line). The mean prestorage concentrations of RDP, RDP_{ND}, and RDP_{NDS} (weighted on the basis of the number of treatments from the intermediate-moisture and high-moisture harvests) were 74.8, 14.2, and 60.6% of CP, respectively, which correspond generally to $\Delta RDP = 0$, $\Delta RDP_{ND} = 0$, and $\Delta RDP_{NDS} = 0$ on the y-axis. Effective degradability was calculated on the basis of a ruminal passage rate of 0.060/h.

ous heating, reaching a minimum of −24.6 percentage units of CP. Overall, this response was best fit by a cubic model with a high coefficient of determination ($R^2 = 0.891$). In general, this relationship suggests that decreasing estimates of RDP commonly associated with spontaneous heating occur largely as a result of reduced contributions from the pool of CP that is soluble in neutral detergent. However, within this study, this generalization is somewhat confounded because severely heated hays exhibited sharply increased concentrations of NDICP (Figures 2A and 2B). Furthermore, a substantial portion of the NDICP pool within severely heated hays was either soluble in water or was associated with forage particles possessing altered physical characteristics that permitted passage through Dacron bags upon machine rinsing (Figures 7A and 7B). In either case, this portion of the NDICP pool in severely heated hays is assumed to be completely rumen degradable within the context of calculations by the equations of Ørskov and McDonald (1979). However, it remains unclear whether this response is biologically relevant, and it is likely dubious to assume that it is. More likely, it suggests that the severity of heating within baling treatments exceeds the reliable limits of the in situ method in predicting in vivo responses.

CONCLUSIONS

Alfalfa-orchardgrass hays exhibited increased concentrations of both NDICP and ADICP in response to spontaneous heating during storage. Although specific regression models varied, each described a curvilinear relationship that was associated closely with HDD or MAX ($R^2 \geq 0.716$). The regression of ΔNDICP on HDD became asymptotic at +24.9 percentage units of CP, indicating there were enormous increases in concentrations of NDICP in our most severely heated hays. Within ranges of heating most commonly encountered under field conditions, estimates of K_d and RDP decreased in close association with measures of spontaneous heating, which is consistent with past work. Interpretation of results was complicated by poor recovery of NDICP from our most severely heated hays following machine rinsing of 0-h Dacron bags; this lost pool of NDICP is by definition entirely degradable in the rumen. As a result, the mean ΔRDP for the 4 most severely heated hays was only −2.6 percentage units of CP, which was a minimal reduction from prestorage controls. It remains unclear whether these responses could be corroborated by other techniques, or whether the severity of these heating increments exceeds the reliable limits for estimating RDP via in situ methodology.

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